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Separation and purification of Arabinogalactan obtained from *Larix gmelinii* by macroporous resin adsorption

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Abstracts: Arabinogalactan (AG) obtained from *Larix gmelinii* R. was purified with the method of macroporous resin adsorption. Effects of various parameters on the adsorption, including adsorption time and temperature, the concentration and the dosage of raw AG, the reused numbers of resin, were investigated. The effect of purification was tested through the removal rate of impurity and the contents of AG and impurity. The optimal condition was determined as follows: adsorbed at 30°C for 2 h with the concentration of raw AG <0.1 g·mL^{-1} and its dosage < 7 mL, the dose of resin was 3 g and reused for 4 times. On the basis of these, macroporous resin column was used for AG purification. The result showed that the AG yield could reach 68.28% with sugar content of 95.02%. The analysis of IR and UV showed that the effect of macroporous resin characteristics on the purification of AG was significant. The obtained product had the same functional groups with standard sample.

Keywords: Arabinogalactan; *Larix gmelinii* R.; Macroporous resin

Introduction

Arabinogalactan (AG) is a kind of water-soluble polysaccharides with strong biologic activity, which is characterized by a limited proportion of lipophilic groups, such as long-chain hydrocarbon groups, attached covalently to free hydroxyl groups. The lipidated arabinogalactans are water soluble and biocompatible and are applied widely in biomedical domains. AG has obvious effects on enhancing immunity (Chintalwar *et al*. 1999), diminishing inflammation (Singha *et al*. 2003), resisting hypersusceptibility, and improving the vim of natural killer cell and macrophage (Richards 2001; Currier *et al*. 2003). AG has a restraining effect on tumor induced by carcinogen while combined with other medicine (Moretao *et al*. 2004). Therefore, AG is not only a type of functional genes of health food but also an important medical material (Nunes *et al*. 2002; Tanaka *et al*. 2003).

At present, larch is the main materials used to extract AG. It is reported that the content of AG in larch is nearly 15%–25%. However, AG obtained from larch contains polyphenolic impurity (Lu 1993). The separation and purification of rude AG became very important steps to obtain AG with high purity. Macroporous adsorptive resin, a kind of organic high polymer absorbent with good adsorption performance, has been developed quickly in recent decades. It has been widely used for the separation and purification of the effective composition with high stability, unique adsorption selectivity, advantage, regeneration, low cost, and superior feasibility in industrial application (Ma 1997). Macroporous adsorptive resin shows an excellent adsorptive capability on polyphenolic material in recent research (Ma 1997). In this paper, an exploratory experiment on the purification of AG from *Larix gmelinii* R. using macroporous adsorptive resin was developed and a sound result was obtained.

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Methods

Extraction of rude AG

Larix gmelinii. samples come from Daxing'an Mountains forest area of Heilongjiang Province, China. AG was extracted by ultrasonic method, followed by filtrated using ultrafiltrated membrane, and the product was condensed and dried by freezing method (Huang 2004). The adsorption effect was analyzed by determining the maximal absorption at 280–285 nm at different adsorption conditions.

Adsorption by macroporous resin

250 g of H macroporous resin (H-MAR) together with 500 mL of alcohol was added into a soxhiet extractor and mixed at 353 K for 12 h. The obtained H-MAR was washed with water when the color of alcohol solution changed from white turbidity to clear.

Regeneration of macroporous resin

The resin was washed by 70% alcohol directly until the color of alcohol changed little after used. The resin was washed by 5% of NaOH or 5% of HCl firstly, and then washed by 70% alcohol, further washed by water until a neutral pH was reached.

Preparation of macroporus resin chromatographic column

Rude AG was purified by chromatographic column with a size of

75 cm × 2 cm using H-MAR as fixed phase, and water as mobile phase. The outflow was collected every two minutes. The content of AG in the outflow was measured by the method of Phenol-Sulfurie Acid (Huang *et al*. 2004, 2005).

Results

AG from *L. gmelinii*. was purified with macroporous resin, and the purification effect was examined through the elimination rate of impurity, the content of AG, and the content of impurity. Finally, the optimal condition of AG purification was determined.

Effects of adsorption conditions

The influence of adsorption time on adsorption effect was analyzed by determining the maximal absorption at 280–285 nm at different adsorption times. The results are shown in Fig. 1.

The elimination rate of impurity increased significantly when the adsorption time was less than 2 h, while it only enhanced 0.13% from 51.78% to 52.46% as the adsorption time varied from 2 h to 4 h. When the adsorption time was prolonged to 6 h, the elimination rate hardly changed, and kept a balance of adsorption.

As the adsorption time was 2 h, the elimination rate of impurity was determined by the changing adsorption temperature, and other conditions were same. The results are shown in Fig. 2. A relatively constant elimination rate of impurity was observed as the temperatures varied from 20°C to 50°C, and it dramatically dropped with further increase of temperatures.

Five milliliter rude AG with different concentrations was loaded into a conical flask containing 3-g H-MAR, and then shaken at a certain temperature. As shown in Fig. 3, when the concentration of AG increased from 0.001 g·mL^{-1} to 0.1 g·mL^{-1}, the removal rate of impurity had no significant changes. The removal rate of impurity was reduced with the increase of the concentration of rude AG. The proper concentration of rude AG should be determined as 0.001 g·mL^{-1} to 0.1 g·mL^{-1}.

The effect of the volume of rude AG solution on adsorption is shown in Fig. 4. The removal rate of impurity was dramatically reduced with the increased volume from 3 mL to 7 mL. However, it kept constant with further increase.

Fig. 5 shows removal rate of impurity is a function of resin (H-MAR) content. The removal rate of impurity enhanced quickly with the increase of resin content in the range of 1–3 g, then kept constant with further increased resin content. This indicated that the adsorption might reach a balance at this stage. These results showed that the purification was improved limited by the increase of resin content.

Resin can be reused for several times after washing by distilled water. The optimum adsorptive conditions determined in the previous experiments were applied in this study. The reused numbers of the resin were changed. The removal rate of impurity decreased slightly when the reused times less than 4 (Fig. 6), whereas, it decreases greatly when the resin is reused for 5 times. It is concluded that the resin could be reused for 4 times.

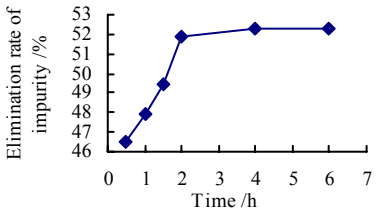


Fig.1 Effects of time on elimination rate of impurity

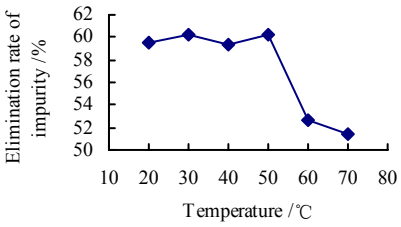


Fig.2 Effects of temperature on elimination rate of impurity

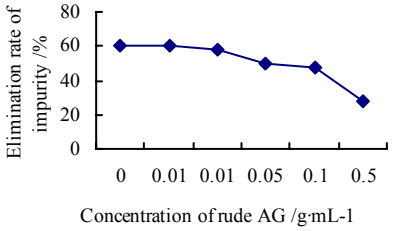


Fig.3 Effects of concentration on elimination rate of impurity

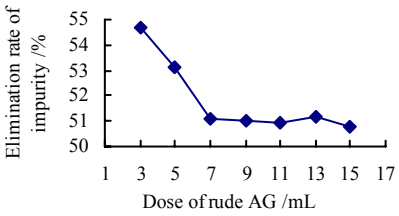


Fig.4 Effects of AG volume on elimination rate of impurity

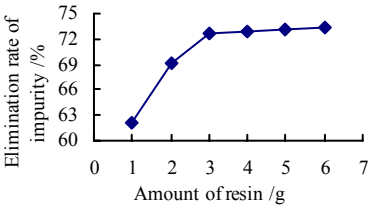


Fig.5 Effect of resin dose on elimination rate of impurity

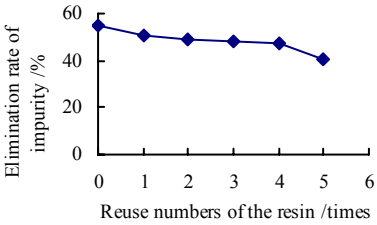


Fig.6 Effect of resin cycles on elimination rate of impurity

Purification of AG by column chromatography with macroporus resin

The contents of AG and its impurity varying with the time are presented separately in Fig. 7 and Fig. 8.

When the eluted time was between 2 min and 68 min, the content of the eluted AG had a narrow distribution. While the elution contained only a little AG as the eluted time was between 68 min and 178 min. When the eluted time was over 178 min, no AG was detected in the elution.

The elution after 178 min was collected, followed by condensation and precipitation using alcohol to obtain the solid AG. The yield was 68.28% and the purity was 95.02%.

Result of IR and UV

IR spectra of AG are shown in Fig. 9. The band positions and corresponding assignments are listed in Table 1. The band at 3 423 cm^{-1} seems to be the characteristic of OH groups of AG which was strong and sharp. Whereas the OH vibration of water

was very broad. In this experiment, the samples were prepared under vacuum to eliminate the effect of adsorbed water on KBr and samples spectra. The band at 2 922 cm^{-1} was due to the stretching vibration of methyl or methylene group. The band at 1 641 cm^{-1} was the stretching vibration of aldehyde acid of AG. The band at 1 079 cm^{-1} was strong and sharp; it seemed to be the characteristic of CO groups in AG. The band at 1 079 cm^{-1} indicated that the functional groups of the raw, the purified and the standard AG were the same. As shown in Fig. 10, the UV adsorption in 280–285 nm of the impurity in the purified AG was less distinct than that of raw AG

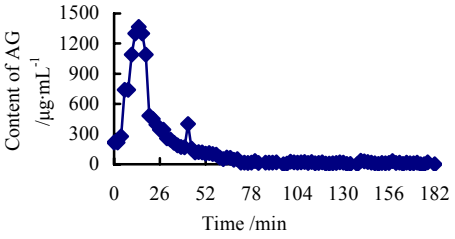


Fig.7 Change of AG content with time

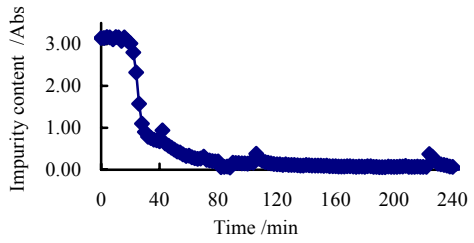
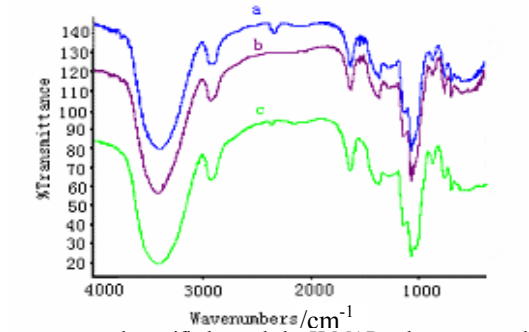


Fig.8 Change of impurity content of AG



a: raw sugar; b: purified sample by H-MAR column; c: standard AG

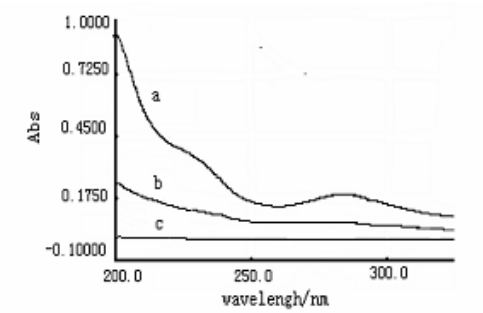
Fig.9 Contrast diagram of IR spectra of five samples

Table 1. Assignment of infra-red absorption of AG samples

Maximum band position (cm^{-1})	Band origin
3423	OH stretching vibration
2922	CH stretching vibration of methyl or methylene group
1641	C=O stretching vibration of aldehyde acid
1376	CH bending vibration of methyl or methylene group
1079	C-O stretching

Conclusion

AG from *Larix gmelinii* R. was purified by H-MAR, the purification effects were tested through examining the removal rate of impurity, the contents of AG and impurity. The optimal condition was determined as follows: adsorptive duration was 2 h, adsorption temperature 30°C, the concentration of rude AG <0.1 $g \cdot mL^{-1}$, the dosage of AG was less than 7 mL, the dose of resin was 3 g, the reused numbers of the resin were 4 times. The yield of AG purified by the macroporous resin column was 68.28 %, and the sugar content was 95.02 %.



a: raw sugar; b: purified sample by H-MAR column; c: standard sugar

Fig.10 Contrast diagram of UV spectra of five samples

IR and UV analysis showed that the effect of H-MAR on the purification of AG was significant. The special functional groups of the product were identical as the standard. Separation and purification of plycan with macroporous resin are promising.

References

Chintalwar, G., Jain, A., Sipahimalani, A., *et al*. 1999. An immunologically active arabinogalactan from *Tinospora cordifolia*. *Phytochemistry,* **52**(6): 1089–1093.

Currier, N.L, Lejtenyi, D., Miller, S.C. 2003. Effect over time of *in-vivo* administration of the polysaccharide arabinogalactan on immune and hemopoietic cell lineages in murine spleen and bone marrow. *Phyomedicine*, **10**(2–3): 145–153.

Huang Zhanhua, Fang Guizhen, Liu Baoliang. 2004. Microwave-assisted extraction of arabinogalactan from *Larix Gemlini* R. wood. *Chemistry and Industry of Forest Products*, **24**: 73–76. (in Chinese)

Huang Zhanhua, Fang Guizhen. 2005. Study on purifying Arabinogalactan from *Larix Gemlini* R. by column chromatography. *Chemistry and Industry of Forest Products*, **25**: 125–128. (in Chinese)

Lu Wenda. 1993. *Larch resource and utilize*. Harbin: Northeast Forestry University Press, p10–11. (in Chinese)

Ma Xiuping, Jiang Chaohui, Yang Yuqin, *et al*. 1997. A study on the adsorption of flavonoids in *Ginkgo biloba* L. Leaves by macroporous adsorptive resins. *China Journal of Chinese Materia Medica*, **22**(9): 539–542. (in Chinese)

Ma Zhanshan. 1997. Application in medicine investigate field of macroporous resin. *Chinese Traditional Patent Medicine*, **19**(12): 40–41. (in Chinese)

Moretao, M.P., Zampronio, A.R., Gorin, P.A.J., *et al*. 2004. Induction of secretory and tumoricidal activities in peritoneal macrophages activated by an acidic heteropolysaccharide (ARAGAL) from the gum of Anadenanthera colubrina (*Angico branco*). *Immunology Letters*, **93**(2–3): 189–197.

Nunes, R.V., Mehansho, H Mellican, R.I, *et al*. 2002. Beverage compositions comprising arabinogalactan and defined vitamins. USA. Pat. 0, 110, 32.

Richards, G.N. 2001. Water soluble lipidated arabinogalactan. USA. Pat. 0, 036, 933.

Singha, P.K., Roy, S., Dey, S. 2003. Antimicrobial activity of Andrographis paniculata. *Fitoterapia,* **74**(7–8): 692–694.

Tanaka, H., Yoshikawa, G., Mukai, K., *et al*. 2003. Process for producing l-arabinose, l-arabinose-containing enzymatically processed products, diet foods, diabetic diet foods and fruit or vegetable juices and process for producing the same. USA. Pat. 0, 040, 489.